JOVE January 25, 2013

17 Sellers Street

Cambridge, MA 02139

Dear Dr. Singh:

Thank you for the opportunity to resubmit the article “Pyrosequencing for Microbial Identification and Characterization” for consideration to be published in the Journal of Visualized Experiments (JOVE). The authors have added our responses to the editorial and reviewer comments below.

Editorial comment: The protocol length is over our three page limit, so please use highlighting to define a total of three pages of protocol to be included in the video.

Authors Response: The three pages have been highlighted in the revised submission.   
  
Editorial Comment: 50405 (resub) - Previous issues (10/4) has been partially addressed. Conditional pass for commercialization, and for required author clarification. \*The Pyromark name has been removed in a few places but is still extensively used (12 times in the 3 pages of protocol).

Authors Response: The Pyromark name has been removed 8 more times from the revised protocol.

Editorial Comment: \*There is a note from the author on Aries: "This submission includes animations that need editing by JOVE. I plan to mail the animation files with time stamps for editing to the JOVE office. Because of the large size of these files, I will mail the files on a USB drive. Please let me know if this is okay to do, and provide the name of the individual who should receive the files." We need more information on what they are asking for before we can agree. Are they asking us to incorporate their own animations into the video, or for us to (re)create animations for them? What sort of animations?

Authors Response: The authors plan to include an animation of the pyrosequencing reaction at the end of the *Introduction* section. The animation will be provided by the author, but we request that JOVE edit the Quick Time movie file to include the times between 0:07 to 1:49. I will attempt to include the file with this resubmission.

Editorial Comment:\*The length is borderline, 3 pages if subheadings and the introductory paragraph are removed. Authors should be careful to take length into consideration if they add material after peer review.

Authors Response: Highlighted text is less than three pages after removing and consolidating text.  
  
Reviewer #1 Comment: I am not clear on the purpose of this article. To me, this work looks like a package insert describing a test, step by step. The authors did not indicate the intent of this work nor the reason of the study.   
  
Authors Response: The purpose of this article is to present the theory and application of the pyrosequencing technology for microbial DNA sequencing and analysis, which has been explained in the *Introduction* and *Results* sections of this submission. The protocol is written step by step to mirror the manufacturer’s instructions to assure proper sample preparation and instrument operation, which will give the highest possible success rate when using this technology.   
  
Reviewer #2 Comment: The abstract should be rewritten to emphasize the findings and conclusions rather than providing a description of the technique and its advantages. The results and discussion section should focus on the sequence data obtained, and how the instrument and technique are useful for rapid identification of mutational changes. There are 11 figures, mostly describing the technique and instrument, but only two figures for the results. The discussion section included three paragraphs that describe the next generation sequencing methods and did not address the findings and their importance in this research.   
  
Authors Response: This submission is not a research or hypothesis based study and therefore this submission did not focus on the results or conclusions from a single organism or DNA sequence. The authors believe the abstract accurately reflects the purpose of this submission.

The purpose of the submission is to present the theory, step-by-step procedure, and analysis of microbial DNA sequencing using the pyrosequencing technology. The discussion section describes other emerging and next generation sequencing technologies besides pyrosequencing, and it did not address a research question or hypothesis.   
  
Reviewer #3:   
I thought this article presented a very clear picture of the techniques involved in performing Pyrosequencing. I could easily operate the instrument with the instructions that were provided. I was pleased to see the antibiotic resistance determination described in the paper. This is a very critical area of medicine right now and the rapid determination of the correct bacterial strain could prevent time wasted on using the incorrect therapeutic intervention. One of the primary reasons for incorrect diagnoses is the time in which it takes to type bacterial pathogens by conventional means.   
  
Authors Comments: None

Regards

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